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Effect of Black Pointed Seed and Vermicompost on Leaf and Seed Infection of Wheat caused by *Bipolaris sorokiniana*

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Abstract

An experiment was conducted to find out the effect of different levels of black pointed seed and vermicompost in soil on leaf blight (*Bipolaris sorokiniana)* severity, yield attributes and seed health status of harvested seed of wheat, cv. Shatabdi. In the field experiment in RCBD design with 18 treatments, the maximum (114.3 and 124.0) emergence of seedling was recorded from apparently healthy seed treated with Provax 200 @ 0.25% sown in soil amended with vermicompost @ 1.5 t ha^{-1} after 7 days and 15 days of sowing, respectively. Leaf blight severity (0-5 scale) on flag leaf and penultimate leaf in flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage were recorded the maximum when 25% black pointed seeds in weight basis were sown without vermicompost. The treatments showed significant variations on number of grains per ear of different severity grades (0-5). Significantly higher 1000 seeds weight (42.70g), grain yield (3.66 t ha^{-1}) and straw yield (4.33 t ha^{-1}) were recorded from the plants germinated from apparently healthy seed treated with Provax 200 @ 0.25% sown in soil with of vermicompost @ 1.5 t ha^{-1} In laboratory experiments, decreasing trend of germination, normal seedling percentage, shoot length, root length, seedling weight and vigor index (VI) of the seedlings emerged from increasing levels of black pointed seed sown revealed that apparently healthy seed treated with Provax 200 @ 0.25% sown with vermicompost @ 1.5 t ha^{-1} and showed promising results in respect of disease severity on leaf and quality seed production.

Keywords: Black point, vermicompost, wheat, seed infection, *Bipolaris sorokiniana*

1. Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world and is a major source of nourishment. It is one of the most important grain crops providing nearly 20% of the total world food requirement. In Bangladesh it is the second major cereal crop next to rice. Though the crop has been introduced in 1961 in the country, it has gained popularity after 1975. In Bangladesh, 4,44,805 ha of land was under wheat cultivation and total production was about 3.031 mt ha^{-1} (BBS, 2016). About one third people of the world one billion as many as 43 countries and provides about 20% of total food calories. It contains carbohydrate (78.1%), Protein (14.7%), minerals (2.1%) and vitamins (Peterson, 1965). However, wheat suffers from about 26 seed

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borne pathogens causing 14 seed borne diseases (Fakir, 1999). Among the diseases, leaf blight caused by *Bipolaris sorokiniana* is the major and devastating disease of wheat in Bangladesh (Hossain and Azad, 1985). The fungi, *B. sorokiniana* is generally considered highly seed transmitted pathogen expressed as black point on seed and possesses a potential threat to the subsequent crops. *Bipolaris* leaf blight appears at the seedling stage (Alam et al., 1994). *Bipolaris sorokiniana* is also a known cause of foliar blight, seedling blight, head blight and common root rot of wheat (Shahzad et al., 2009). The fungi can produce upto 11.67 mm of root lesion in wheat seedlings and 96.67% of leaf segment disease after artificial inoculation (Laila et al., 2010). *Bipolaris sorokiniana* at flag leaf stage may cause of 7-100% reduction in formation of grains/ear (Hossain and Azad, 1994). The pathogen attacks wheat grains causing black point and the disease reduced yield up to 40% in field condition (Hossain et al., 1998), in severe cases it may cause of 57.6% and 64.5% yield reduction of wheat due to *Bipolaris* leaf blight in cultivars shatabdi and sonalika, respectively (Rashid and Fakir, 1998). *Bipolaris sorokiniana* is most frequently associated with poor germination and abnormal seedlings of wheat (Ammara et al., 2001). Virtually, it is impossible to produce black point free seed; however, seed with a reduced level of black point can be produced that may ensure good germination and high seedling vigor (Aulakh et al., 1988). Seed health plays an important role for successful cultivation and yield exploitation of a crop species. Among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination, but also reduce seed vigor resulting in low yield. Seed-borne pathogens of wheat are responsible to cause variation in plant morphology and also reducing yield up to 15-90% if untreated seeds are grown in the field (Wiese, 1984).

Organic fertilizers are the key to improve the sustainability of agricultural farming system and soil productivity. Vermicompost helps in increasing the organic matter content of soil, which helps in increasing the natural productivity of soil. It is a viable technology for converting waste into organic fertilizers (Gutierrez-Micelietet al., 2011) endowed with hormone- like substances. The vermicomposting is bio-oxidation and stabilization of organic material involving the joint action of earthworm and microorganisms. Although, microbes are responsible for the biological degradation of the organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering biological activity (Aira et al., 2002). The combination of inorganic fertilizers and vermicompost increased different parameters leading to bulb yield of onion and vermicompost soulfully resulted in maximum total soluble solid (Mandal et al., 2013). Use of vermicompost reduces the cost of production, increases plant's health and resistance against biotic and abiotic causes and fertility and water holding capacity of the soil. Nesterenko et al. (2009) found that the lowest wheat disease incidence caused by *Bipolaris sorokiniana* in two crop plantings values were obtained from the second planting in soil like substrate (SLS) obtained by sequential wheat straw treatment with a fungus (*Pleurotus ostreatus*) and worms (*Eisenia foetida*). The values were 26% and 41% at the first and the second infection levels, respectively. For soil the values were 60% and 82%, respectively, and for sand they were 67% and 74%, respectively. SLS significantly suppressed the germination of *B. sorokiniana* conidia. Seed infection level had a significant effect on disease incidence and severity at different growth stages like flag leaf stage, panicle initiation stage, flowering stage, milk stage and hard dough stage (Chowdhury et al., 2010). Soil amendment with vermicompost can be the eco-friendly approach to control the leaf blight disease.

Considering the above facts, the present research program has been designed to determine the effect of different levels of black pointed seed and vermicompost on leaf blight (*Bipolaris sorokiniana*) severity, yield attributes and seed health status of harvested seed of wheat. The objectives of the present study were (i) to evaluate the effect of different levels of black pointed seeds and vermicompost on leaf blight severity of wheat, (ii) to determine the effect of different levels of black pointed seeds and vermicompost on plant growth parameters of wheat, (iii) to estimate the effect of different levels of black pointed seeds and vermicompost on yield and seed health status of harvested seed of wheat.

2. Materials and Methods

2.1. Field experiment

The field experiment was conducted at the farm field allotted for the Department of Plant Pathology during the Rabi season from November 2013 to April 2014.Wheat seed samples of variety Shatabdi was collected from Santhia, Pabna. The collected seed sample was physically sorted out to separate apparently healthy looking seeds with bold golden color and black pointed seeds. Then the black pointed seeds and apparently healthy looking seeds were mixed in different percentages of weight basis to prepare different treatments. However, Vermicompost was produced by using vermicomposting "chari" method. In this method, 75% cow dung and 25% kitchen wastes were subjected to produce vermicompost by the decomposition of organic wastes facilitated through earthworm *Eisenia fetida.* The seed samples were sown in field plots amended with different amount of vermicompost. There were 18 treatments. T_1 (Apparently healthy seed treated with Provax 200 @ 0.25%), T_2 (5% black pointed seed in weight basis), T_3 (10% black pointed seed in weight basis), T_4 (15% black pointed seed in weight basis), T_5 (20% black pointed seed in weight basis), T_6 (25% black pointed seed in weight basis), T_7 (Vermicompost @1.5 t ha^{-1}+T_1), T_8 (Vermicompost @ 1.5 t ha^{-1}+T_2), T_9 (Vermicompost @ 1.5 t ha^{-1}+T_3), T_{10} (Vermicompost @ 1.5 t ha^{-1}+T_4), T_{11} (Vermicompost @ 1.5 t ha^{-1}+T_5), T_{12}

(Vermicompost @ 1.5 t ha^{-1}+T_6), T_{13} (Vermicompost @ 3.0 t ha^{-1}+T_1), T_{14} (Vermicompost @ 3.0 t ha^{-1}+T_2), T_{15} (Vermicompost @ 3.0 t ha^{-1}+T_3), T_{16} (Vermicompost @ 3.0 t ha^{-1}+T_4), T_{17} (Vermicompost @ 3.0 t ha^{-1}+T_5), T_{18} (Vermicompost @ 3.0 t ha^{-1}+T_6). The experiment was laid out in RCBD design with three replications. The experiment was divided into three blocks. Blocks represented the replication. So the total number of unit plots in the entire experiment was 3×18=54. Size of each unit plot was 1.5 m×1 m=1.5 m^2. The distance between sub plot was 0.5m and block was 1m. The field was fertilized at the rate of 220 kg Urea, 180 kg TSP, 50 kg MP, 120 kg Gypsum and 10 t Cow dung ha^{-1} (Krishi Projukti Hatbooi, 2005). Two third of Urea, full dose of TSP, MP, Gypsum and Cow dung was applied at the time of final land preparation. Remaining one third of Urea was applied at 21 days after seed sowing. Wheat seeds were sown in the field at the rate of 120 kg ha^{-1}. The seeds were placed continuously in lines properly at a depth of 5cm and were covered by soil with the help of land. The distance between the lines was 25 cm. The weeds were controlled by nirani (hand hoe). After sowing, light irrigation was given for proper germination. Then flood irrigation was given at crown root initiation (20 DAS), heading (50 DAS) and grain filling (70 DAS) stages. Randomly 10 plants were selected and tagged for rating and mean values were determined to get rating score of each treatment. Disease severity of Flag leaf and Penultimate was measured by using the following "0-5" scale (Horsfall and Barratt, 1945) at five growth stages of plant viz. flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage. The Leaf blight severity of the disease was recorded following 0-5 grade (Table 1).

Table 1: Disease severity grades (0-5) according to Horsfall and Barratt (1945)

% LAD	Scale/grade
0	0
0.1-5	1
5.1-12	2
12.1-25	3
25.1-50	4
>50	5

Percent Disease Index (PDI) of Flag leaf and Flag leaf-1 was measured by using the following formula (Horsfall and Barratt, 1945) at five growth stages of plant viz. flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage.

PDI=(Sum of total ratings/grading)/(Total No. of observation ×Highest Grade in the scale)×100

The disease severity grade of harvested seeds was done following the 0-5 rating scale (Gilchrist, 1985). The rating scale is as follows:0 (Free from infection), 1 (Only embryo blackish), 2 (Embryo and its adjacent area slightly infected), 3 (Embryo and less than ¼of grains are discolored), 4 (Embryo and ½ of grain are infected), 5 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored).The crop was harvested at full ripening stage on 30 March, 2014.

2.2. Laboratory experiment

The laboratory experiment was conducted in the Molecular Plant Pathology Laboratory of Department of Plant Pathology. Sher-e-Bangla Agricultural University, Dhaka during the period of April 2014 to July 2014. For studying the seed health, blotter method and paper towel methods were used following CRD with three replications.

2.2.1. Blotter test

To determine the seed health status, the blotter method (ISTA, 1996) was used. 400 seed were tested for each treatment. In this method, three layers of blotter paper were soaked in sterilized water and placed at the bottom of each sterilized glass petridish. Then, twenty five seeds were plated on the blotter paper in a petridish maintaining equal distance and covered with lid. The seeds on petridishes were incubated in an air cooled room at about 20 °C temperature under 12/12 hr light and darkness cycle for 7 days. Sterilized water was added time to time to maintain the moisture. After 7 days of incubation, the seeds were observed for the presence of seed-borne *Bipolaris sorokiniana* under stereo binocular microscope. Germination of the seeds was also being recorded.

3.2.2. Rolled paper towel method

Seedling infection and seedling vigor test were done by following the Rolled Paper Towel Method (Warham, 1990). In this method, one hundred and fifty seeds were randomly taken from each treatment. 50 seeds were placed uniformly between a pair of moist paper towels. The towels were rolled and the two ends were closed with rubber bands. Then the rolled papers containing seeds were placed in an upright position for 7 days at room temperature under normal 12/12 light and darkness cycle. After incubation period, observations pertaining to germination, number of normal seedlings, number of abnormal seedlings and number of dead seeds were recorded. The normal and abnormal seedlings were categorized according to ISTA rules (1996). Twenty seedlings were randomly selected from each paper and their individual shoot and root length will be measured. The shoot and root portions were blotted dry with fine tissue paper and fresh weight will be taken. Vigor of the seedling was determined by the following formula (Baki and Anderson, 1972):

Vigor Index = (Mean of root length+Mean of shoot length)×% Seed germination

2.3. Analysis of data

The collected data for different parameters were compiled and tabulated in proper form. Appropriate statistical analysis was made by MSTAT Computer Package program. The treatment means were compared by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Field experiment

Considering disease incidence at 7 DAS and 15 DAS of wheat seedlings,the treatments differed significantly (Table 2). The number of infected seedling safter 7 DAS was the maximum in T_7 (114.3) whereas seedlings were the least infected in T_6 (89.0). The similar trend was found in case of number of infected seedlings after 15 DAS. Seedlings infected by *Bipolaris sorokiniana* were the highest in number /m^2 in T_7 (124.0). The present result is supported by Hossain (2000) who reported that black point infection greatly affected seed germination and seedling emergence of wheat and percent reduction in germination become higher with the increase level of black pointed seed. These findings were also supported by other researchers (Machacek and Graney, 1938). Machacek and Graney (1938) who reported that seed infected with *Helminthosporium sativum* produced only 24.8% plant stand and resulted 80.6% seedling infection. Edwards and Burrows (1988) reported that vermicompost consistently improved seed germination, enhanced seedling growth and development, and increased plant productivity significantly. Application of vermicompost in potted and field crops displayed excellent growth performances in terms of height of plants, colour and texture of leaves, appearance of fruiting structures etc. as compared to chemical fertilizers.

Significant variations were found in leaf blight severity on Flag leaf in different growth stages- flag leaf, panicle initiation, flowering stage, milking stage and hard dough stage (Table 3). During flag leaf stage, no disease occurred in T_7 and T_{13} whereas the maximum (5.33%) disease severity was found in T_6. The maximum (10.00%) disease severity value was found in T_6 at panicle initiation stage. On the contrary, T_7 showed minimum (1.00%) disease severity which was statistically similar with T_{13} (1.67%). Similar trend of disease severity was observed at flowering stage also. During milking stage, T_6 (40.67%), T_5 (37.33%) and T_4 (36.00%) were statistically similar in causing maximum disease severity. On the other hand, the minimum (20.00%) disease severity was found

Table 2: Effect of different levels of black pointed seed and vermicompost on disease incidence at 7 DAS and 15 DAS

Treatment	No. of seedlings m^{-2}	
	7 DAS	15 DAS
T_1	102.3^{c-f}	110.7^{b-h}
T_2	100.7^{d-h}	109.7^{c-h}
T_3	96.00^{f-i}	107.0^{e-h}
T_4	94.33^{g-i}	104.3^{f-h}
T_5	$93.00^{h,i}$	$102.0^{g,h}$
T_6	89.00^{i}	100.7^{h}
T_7	114.3^{a}	124.0^{a}
T_8	109.0^{a-c}	$121.0^{a,b}$
T_9	106.7^{a-e}	118.3^{a-d}
T_{10}	103.0^{c-f}	117.7^{a-e}
T_{11}	102.3^{c-f}	114.0^{a-f}
T_{12}	101.3^{c-g}	111.7^{b-h}
T_{13}	$112.0^{a,b}$	120.7^{a-c}
T_{14}	108.3^{a-d}	117.3^{a-e}
T_{15}	105.3^{b-e}	115.3^{a-f}
T_{16}	104.0^{c-e}	112.0^{b-g}
T_{17}	101.0^{d-g}	110.7^{b-h}
T_{18}	100.3^{e-h}	107.7^{d-h}
LSD (0.01)	7.77	11.04

Table 3: Effect of different levels of black pointed seed and vermicompost on leaf blight severity (0-5) scale on Flag leaf of wheat at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage

Treat-ment	Disease severity in different growth stages at Flag leaf (%)				
	Flag leaf stage	Panicle initiation stage	Flow-ering stage	Milking stage	Hard dough stage
T_1	0.67^{i}	$2.33^{h,i}$	$4.00^{i,j}$	28.67^{e-h}	41.33^{e-g}
T_2	1.33^{h}	3.67^{g}	$6.33^{f,g}$	30.67^{c-g}	44.00^{d-f}
T_3	2.67^{f}	$6.00^{d,e}$	8.00^{e}	34.00^{b-d}	48.00^{b-d}
T_4	2.67^{f}	7.33^{c}	10.00^{d}	$36.00^{a,b}$	$50.67^{b,c}$
T_5	4.00^{c}	8.67^{b}	$11.67^{b,c}$	$37.33^{a,b}$	$54.00^{a,b}$
T_6	5.33^{a}	10.00^{a}	14.00^{a}	40.67^{a}	58.67^{a}
T_7	0.00^{j}	1.00^{j}	2.67^{k}	20.00^{j}	32.67^{h}
T_8	0.67^{i}	$1.67^{i,j}$	$4.67^{h,i}$	24.67^{h-j}	$35.67^{g,h}$
T_9	1.33^{h}	$2.00^{h,i}$	$5.33^{g,h}$	27.33^{g-i}	43.33^{d-f}
T_{10}	2.00^{g}	4.67^{f}	6.67^{f}	30.67^{c-g}	41.33^{e-g}
T_{11}	2.00^{g}	$5.33^{e,f}$	8.33^{e}	33.33^{b-e}	44.00^{d-f}
T_{12}	3.00^{e}	6.33^{d}	$10.67^{c,d}$	$34.67^{b,c}$	46.00^{c-e}
T_{13}	0.00^{j}	$1.67^{i,j}$	$3.33^{j,k}$	$23.00^{i,j}$	$36.00^{g,h}$
T_{14}	2.00^{g}	2.67^{h}	$5.33^{g,h}$	27.67^{f-i}	$39.33^{f,g}$
T_{15}	1.33^{h}	4.67^{f}	$7.33^{e,f}$	29.33^{d-h}	43.33^{d-f}
T_{16}	2.67^{f}	$5.67^{d,e}$	9.67^{d}	32.67^{b-f}	46.67^{c-e}
T_{17}	3.33^{d}	$6.00^{d,e}$	10.00^{d}	$35.33^{b,c}$	48.67^{b-d}
T_{18}	4.67^{b}	8.33^{b}	12.00^{b}	$36.00^{a,b}$	$50.67^{b,c}$
LSD (0.01)	0.32	0.84	1.27	5.01	6.24

in Treatment T_7 statistically similar with T_8 (24.67%) and T_{13} (23.00%). During hard dough stage, the maximum (58.67%) value was found in T_6 (58.67%) which was statistically similar with T_5 (54.00%). On the other hand, the minimum (32.67%) disease severity was observed in Treatment T_7 which was statistically similar with T_8 (35.67%) and T_{13} (36.00%).

Disease severity on Penultimate leaf also showed significant variations in different stages-flag leaf, panicle initiation, flowering, milking and hard dough (Table 4). Disease severity was the maximum in T_6 where as it was the minimum in T_7 (2.00%). Significant variations were found in leaf blight severity in panicle initiation stage on Penultimate leaf. During panicle initiation stage, the maximum (12.00%) disease severity was in Treatment T_6, statistically similar with T_{18} (10.00%) and T_5 (9.67%). On the other hand, the minimum (2.67%) disease severity was in T_7 statistically similar with by T_8 (3.00%) and T_{13} (3.33%). During flowering stage, the maximum disease severity was (15.67%) in T_6.

Table 4: Effect of different levels of black pointed seed and vermicompost on leaf blight severity (0-5) scale on Penultimate leaf of wheat at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage

Treatment	Disease severity in different growth stages at Penultimate Leaf (%)				
	Flag leaf stage	Panicle initiation stage	Flowering stage	Milking stage	Hard dough stage
T_1	2.00^{h}	4.67^{g}	6.67^{h,i}	32.67^{d-g}	52.67^{c-f}
T_2	4.00^{e}	6.33^{f}	8.00^{g}	34.67^{b-g}	55.00^{b-e}
T_3	4.00^{e}	7.67^{d,e}	9.33^{e,f}	36.00^{b-f}	58.00^{a-d}
T_4	4.00^{e}	8.00^{c,d}	11.33^{d}	40.00^{a-c}	60.67^{a-c}
T_5	4.67^{d}	9.67^{b}	13.33^{b,c}	41.33^{a,b}	62.67^{ab}
T_6	7.33^{a}	12.00^{a}	15.67^{a}	44.67^{a}	65.33^{a}
T_7	2.00^{h}	2.67^{h}	4.66^{k}	25.67^{h}	41.33^{g}
T_8	2.67^{g}	3.00^{h}	5.33^{j,k}	28.33^{g,h}	44.00^{f,g}
T_9	2.67^{g}	4.33^{g}	6.00^{i,j}	32.00^{e-h}	46.67^{e-g}
T_{10}	3.33^{f}	6.33^{f}	7.33^{g,h}	34.00^{c-g}	48.00^{e-g}
T_{11}	4.67^{d}	7.00^{e,f}	9.67^{e}	36.00^{b-f}	50.67^{d-g}
T_{12}	5.33^{c}	8.67^{c}	11.33^{d}	37.33^{b-e}	52.00^{c-f}
T_{13}	2.00^{h}	3.33^{h}	5.33^{j,k}	28.33^{g,h}	47.33^{e-g}
T_{14}	2.00^{h}	4.67^{g}	6.33^{h-j}	30.33^{f-h}	47.67^{e-g}
T_{15}	3.33^{f}	6.67^{f}	8.33^{f,g}	33.67^{c-g}	49.67^{d-g}
T_{16}	4.67^{d}	7.67^{d,e}	10.00^{e}	35.67^{b-f}	52.33^{c-f}
T_{17}	5.33^{c}	8.67^{c}	12.67^{c}	37.00^{b-f}	54.00^{b-e}
T_{18}	6.00^{b}	10.00^{b}	14.00^{b}	39.33^{a-d}	58.00^{a-d}
LSD (0.01)	0.52	0.99	1.13	6.83	9.62

On the other hand, the minimum disease severity (4.66%) was in T_7 which was statistically similar with T_8 (5.33%) and T_{13} (5.33%). During flowering stage, the maximum(44.67%) disease severity was in T_6 statistically similar with T_5 (41.33%) and T_4 (40.00%). On the other hand, the minimum disease severity (25.67%) was observed in T_7 statistically similar with T_8 (28.33%) and T_{13} (28.33%). During hard dough stage, the maximum disease severity was (65.33%) in T_6 statistically similar with T_5 (62.67%). On the other hand, the minimum disease severity (41.33%) was observed in T_7 statistically similar with T_8 (44.00%).

The disease severity was found to increase with the age of plant and the maximum disease severity was observed in hard dough stages in all treatments than the other stages. Leaf blight development is a usual consequence of the seed to plant to seed transmission of the pathogen *Bipolaris sorokiniana* under field condition (Rashid, 1996; Rashid and Fakir, 1998). Moreover, higher the level of seed borne fungal infection, there should be higher primary inoculums level in the field. So, higher level of black pointed seed resulted with higher infection in the field in the present experiment. The present findings were well supported by previous researchers (Nema and Joshi, 1974; Hossain and Azad, 1992; Malakar, 2003; Hossain, 2000; Reza et al., 2006). Nema and Joshi (1974) reported that age was one of the important factor influencing disease intensity and susceptibility of wheat plant to *H. sativum*. Hossain and Azad (1992) reported that age of crop plant resulted higher incidence of leaf spot caused by *B. sorokiniana*. Malakar (2003) found that different grade of black point affected seeds caused significant variation in leaf blight development in adult plant. Reza et al. (2001) also reported that blight severity in adult plants of wheat increased with the increase in level of *Bipolarissorokiniana* infected seeds. Temperature is also is an important factor for leaf blight incidence. Hossain (2000) reported that temperature 25-28 °C normally prevails in March when wheat plant turns to soft dough to hard dough stage. Therefore, maximum leaf blight disease incidence occurs at that time. The result of the present investigation is also supported by Hossain (2000) who found that significant leaf severity of wheat at flowering and milk ripening stages under field condition when different black point infected seeds were sown.

The number of grains under grade-0 was found in a range of 34.27 to 45.50 (Table 5). The maximum (45.50) count of healthy seeds was observed in T_7 which was statistically similar with T_8 (44.13) and T_{13} (43.03) whereas the minimum (34.27) count of healthy seeds was recorded in T_6. The number of grains under grade-1 was found the highest (3.73) in T_6. However, the minimum (3.23) number of seeds was recorded in T_7. The number of grains under grade-2 was the highest (0.45) in T_6 whereas the minimum (0.12) number of seeds was recorded in T_7. The number of grains under grade-3 was the highest (0.55) in T_6 and T_{18} where

Table 5: Effect of different levels of black pointed seed and vermicompost on number of grains/ear of different severity grades (0-5) of harvested seeds of wheat

Treat-ment	No. of different grade seeds					
	0	1	2	3	4	5
T_1	41.13^{c,d}	3.40^{c-f}	0.19^{f,g}	0.32^{g,h}	0.53^{g,h}	0.80^{h-k}
T_2	39.67^{d,e}	3.43^{b-f}	0.24^{e,f}	0.36^{f,g}	0.53^{g,h}	0.87^{f-i}
T_3	37.00^{f-h}	3.57^{a-c}	0.23^{e,f}	0.40^{e,f}	0.60^{e,f}	0.96^{c-e}
T_4	35.00^{h,i}	3.57^{a-c}	0.37^{b-d}	0.46^{c,d}	0.65^{c-e}	1.07^{a,b}
T_5	34.83^{h,i}	3.63^{a,b}	0.40^{a-c}	0.50^{a-c}	0.73^{a}	1.13^{a}
T_6	34.27^{i}	3.73^{a}	0.45^{a}	0.55^{a}	0.73^{a}	1.13^{a}
T_7	45.50^{a}	3.23^{f}	0.12^{h}	0.23^{i}	0.45^{i}	0.73^{k}
T_8	44.13^{a,b}	3.27^{e,f}	0.19^{f,g}	0.30^{h}	0.50^{h,i}	0.80^{i-k}
T_9	42.33^{b,c}	3.30^{d-f}	0.25^{e}	0.35^{f-h}	0.55^{f,g}	0.83^{g-j}
T_{10}	41.50^{c,d}	3.40^{c-f}	0.33^{d}	0.41^{d,e}	0.60^{e,f}	0.88^{e-i}
T_{11}	39.17^{d-f}	3.49^{b-e}	0.36^{c,d}	0.46^{b,c}	0.66^{c,d}	0.95^{c-f}
T_{12}	38.50^{e-g}	3.55^{a-c}	0.40^{a-c}	0.50^{a-c}	0.70^{a,b,c}	0.98^{b-d}
T_{13}	43.03^{a-c}	3.30^{d-f}	0.15^{g,h}	0.23^{i}	0.47^{i}	0.76^{j,k}
T_{14}	41.13^{c,d}	3.43^{b-f}	0.21^{e,f}	0.32^{g,h}	0.53^{g,h}	0.82^{h-k}
T_{15}	39.62^{d,e}	3.46^{b-f}	0.26^{e}	0.39^{e,f}	0.55^{f,g}	0.89^{e-h}
T_{16}	37.26^{e-h}	3.51^{a-d}	0.33^{d}	0.48^{b,c}	0.62^{d,e}	0.92^{d-g}
T_{17}	36.50^{g-i}	3.63^{a,b}	0.38^{b-d}	0.51^{a,b}	0.68^{b,c}	0.97^{c-e}
T_{18}	35.29^{h,i}	3.66^{a,b}	0.42^{a,b}	0.55^{a}	0.73^{a,b}	1.03^{b,c}
LSD (0.01)	2.50	0.23	0.05	0.05	0.05	0.09

as the minimum (0.23) number of seeds was in T_7 and T_{13} (0.23). The number of grains under grade-4 was the highest (0.73) in T_6 which was statistically similar with T_{18} (0.73) and T_5 (0.73). The lowest (0.45) number of seeds was recorded in T_7 which was statistically similar with T_{13} (0.47) and T_8 (0.50). The number of grains under grade-5 was the highest (1.13) number of seeds was observed in T_6 and T_5 (1.13) which was statistically similar with T_4 (1.07). The lowest (0.73) number of seeds was recorded in T_7 statistically similar with T_{13} (0.76). These findings were well supported by Rashid (1996); Rashid and Fakir (1998); who reported that development of black point infection in the field was due to seed to plant to seed transmission of black point pathogen. Hossain (1998) observed that leaf infection at flowering and milking stages has direct effect on the reduction of formation of healthy grains with the increase in number of black pointed as well as discolored grains. Hossain (2000) also reported significant relationship of leaf blight severity with grain infection. Reza et al. (2006) reported that 65.36% disease severity the corresponding 17.42% seed infection.

Significant variations were observed among the treatments in respect of 1000 seeds weight, grain yield and straw yield of wheat (Table 6). The 1000 seeds weight (g) of wheat ranged from 37.42 to 42.70 g. The highest (42.70) weight of 1000 seeds was found in T_7 which was statistically similar with T_{13} which provided 41.91 g weight of 1000 seeds. The lowest (37.42) weight of 1000 seeds was found in T_6 which is statistically similar with T_5 which provided 38.02 g weight of 1000 seeds. Considering grain yield (t ha^{-1}) ranged from 2.33 to 3.66. The highest (3.66) grain yield (t ha^{-1}) was found in T_7 statistically similar with T_8, which provide 3.54. The lowest (2.33) grain yield (t ha^{-1}) was found in T_6 which was statistically similar with T_5 which provide 2.48 (t ha^{-1}). Considering straw yield (t ha^{-1}) ranged from 3.05 to 4.33.The highest (4.33) straw yield (t ha^{-1}) was found in T_7 statistically similar with T_8 and T_{13} which provide 4.21 and 4.13 (t ha^{-1}). The lowest (3.05) straw yield (t ha^{-1}) was found in T_6 statistically similar with T_5 which provide 3.22 (t ha^{-1}). Malakar (2003) reported that there was a decreasing trend in yield and yield contributing characters with increasing in severity of black point infected seeds. Yield reduction due to sowing of black pointed seeds has also been reported by the other workers (Parashar and Chohan 1967); Zwatz, 1975 and Nalli, 1986. Ahmed and Hossain (2005) found the highest 1000 grain

Table 6: Effect of different levels of black pointed seed and vermicompost on 1000 seeds weight and yield of wheat

Treat-ment	No. of different grade seeds		
	1000 seed weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
T_1	41.13^{a-d}	3.20^{c-e}	3.93^{a-d}
T_2	40.34^{b-e}	3.03^{d-f}	3.83^{b-e}
T_3	39.85^{b-f}	2.94^{e-g}	3.68^{c-e}
T_4	38.98^{d-g}	2.68^{g,h}	3.45^{e-g}
T_5	38.02^{f,g}	2.48^{h,i}	3.22^{f,g}
T_6	37.42^{g}	2.33^{i}	3.05^{g}
T_7	42.70^{a}	3.66^{a}	4.33^{a}
T_8	41.81^{a-c}	3.54^{a,b}	4.21^{a,b}
T_9	41.09^{a-d}	3.32^{b-d}	4.07^{a-c}
T_{10}	40.71^{a-e}	3.21^{c-e}	3.96^{a-d}
T_{11}	40.05^{b-f}	3.17^{d,e}	3.84^{b-e}
T_{12}	39.56^{c-g}	2.90^{e-g}	3.63^{c-f}
T_{13}	41.92^{a,b}	3.50^{a-c}	4.13^{a,b}
T_{14}	41.22^{a-d}	3.28^{b-d}	4.02^{a-d}
T_{15}	40.62^{a-e}	3.14^{d,e}	3.91^{a-d}
T_{16}	39.89^{b-f}	3.02^{d-f}	3.79^{b-e}
T_{17}	39.04^{d-g}	2.93^{e-g}	3.60^{d-f}
T_{18}	38.65^{e-g}	2.74^{f-h}	3.46^{e-g}
LSD (0.01)	2.28	0.32	0.45

weight and the highest yield (3.63 t ha^{-1}) in wheat variety kanchan when sprayed with fungicide Tilt-250 EC. Ferdouset al. (2003) found grain yield of wheat was 2880-4000 kg ha^{-1} by using different micronutrients in the field. The results are in accordance with Oppitz and Hoesser (1979) who reported that seed borne pathogens of wheat not only reduced the germination but also affected seedling vigor that resulting in low yield. Baker and Barrett (1994) found that the earthworms (*Aporrectodea trapezoids*) increased growth of wheat crops by 39% and grain yield by 35%. It also reduced crop diseases as compared to the control. Palanisamy (1996) also reported that earth worms and its vermicompost improve the growth and yield of wheat by more than 40%. Bhatia et al. (2000) also studied impact of vermicompost on potted wheat crops and came with very encouraging results.

4.2. Laboratory experiment

In blotter method, the effect of the treatments was significantly different regarding germination percentage and incidence of *Bipolaris sorokiniana* of wheat (Table 7). The maximum seed germination (93.33%) was found in T_7 statistically similar with T_{13} (91.33%). On the other hand, the lowest seed germination (65.33%) was recorded in T_6 statistically similar with T_{18} (70.33 %).*Bipolaris sorokiniana* ranged from 8.667 to 53.67 % where the treatment T_7 (8.667%) was found to have lowest infection preceded by T_{13} (11.33%) and T_1 (13.33%). On the other hand, the highest incidence (53.67%) was recorded in T_6 statistically similar withT_{18} (50.33%).

Table 7: Effect of different levels of black pointed seed and vermicompost on germination, incidence of *Bipolaris sorokiniana*, normal and abnormal seedling % and dead seed % of wheat after harvesting

Treatment	Blotter method		Paper towel method			
	% Germination	% *Bipolaris sorokiniana*	% Germination	% Normal seedling	% Abnormal seedling	% Dead seed
T_1	88.33^{a-d}	13.33^{m-o}	86.67^{a-c}	$72.33^{b,c}$	14.33^{g-i}	$13.33^{j,k}$
T_2	84.67^{a-e}	$18.67^{k,l}$	83.33^{a-e}	67.00^{c-e}	$16.33^{f,g}$	$16.67^{g,h}$
T_3	80.33^{c-g}	$25.33^{i,j}$	77.00^{c-g}	57.33^{f-h}	$19.67^{d,e}$	$23.00^{d,e}$
T_4	75.00^{e-h}	$35.00^{f,g}$	74.00^{d-h}	52.00^{g-i}	$22.00^{c,d}$	24.67^{d}
T_5	71.67^{f-h}	$43.33^{c,d}$	$69.33^{g,h}$	$45.00^{i,j}$	$24.33^{b,c}$	30.67^{b}
T_6	65.33^{h}	53.67^{a}	64.67^{h}	37.33^{j}	27.33^{a}	35.33^{a}
T_7	93.33^{a}	8.667^{o}	90.67^{a}	82.00^{a}	8.667^{k}	9.33^{l}
T_8	90.67^{a-c}	14.67^{l-n}	$88.33^{a,b}$	$77.67^{a,b}$	$10.67^{j,k}$	11.67^{j-l}
T_9	86.00^{a-d}	$19.00^{k,l}$	83.67^{a-d}	71.00^{b-d}	$13.33^{h,i}$	$16.33^{h,i}$
T_{10}	81.00^{b-g}	$28.33^{h,i}$	79.00^{b-g}	63.33^{d-f}	$15.67^{g,h}$	$21.00^{e,f}$
T_{11}	77.67^{d-g}	$37.67^{e,f}$	76.33^{c-g}	$58.00^{f,g}$	$18.33^{e,f}$	24.67^{d}
T_{12}	72.33^{f-h}	$46.33^{b,c}$	70.33^{f-h}	50.00^{g-i}	$20.33^{d,e}$	$29.67^{b,c}$
T_{13}	$91.33^{a,b}$	$11.33^{n,o}$	89.33^{ab}	$76.67^{a,b}$	$12.67^{i,j}$	$10.67^{k,l}$
T_{14}	87.67^{a-d}	$16.67^{l,m}$	86.33^{a-c}	71.00^{b-d}	$15.33^{g,h}$	$13.67^{i,j}$
T_{15}	82.33^{b-f}	$22.67^{j,k}$	80.67^{a-f}	$62.00^{e,f}$	$18.67^{e,f}$	$19.33^{f,g}$
T_{16}	78.67^{d-g}	$31.00^{g,h}$	76.00^{c-g}	56.00^{f-h}	$20.00^{d,e}$	24.00^{d}
T_{17}	73.00^{f-h}	$40.33^{d,e}$	72.33^{e-h}	$49.00^{h,i}$	$23.67^{b,c}$	27.67^{c}
T_{18}	$70.33^{g,h}$	$50.33^{a,b}$	$68.67^{g,h}$	$44.00^{i,j}$	24.67^{b}	31.33^{b}
LSD (0.01)	10.99	5.094	11.26	8.52	2.417	2.914

In rolled paper towel method, wheat seeds samples having different levels of black pointed seed and vermicompost had significant effect on seed germination, normal and abnormal seedling productionand dead seed percentage of wheat in Rolled Paper towel method (Table 7). The germination varied from 64.67 to 90.67%. The highest germination (90.67%) was found in T_7 statistically similar with T_{13} (89.33%) and T_8 (88.33%) and. The lowest (64.67%) was found in T_6 statistically similar with T_{18} (68.67%) and T_5 (69.33%).The treatment showed significant difference from one another regarding percent normal seedling and the results for all the treatments ranged from 82.00 to 37.33% ,where the maximum counts (82.00%) were found in T_7 statistically similar with T_8 (77.67%) and T_{13} (76.67%). The lowest (37.33%) was found in T_6 statistically similar with T_{18} (44.00%) and T_5 (45.00%). The number of abnormal seedlings was found to be increased with the increase of black pointed seeds. In case of abnormal seedlings, they ranged from 8.667 to 27.33% where the highest (27.33%) percent was observed in T_6 followed by T_{18} (24.67%). The lowest (8.667%) was found in T_7 statistically similar with T_8 (10.67%).Percent dead seed was found the minimum 9.33 in T_7 which was statistically similar with T_{13} (10.67%). The highest (35.33%) dead seed was found in T_6 followed by T_{18} (31.33%) and T_5 (30.67%). Similar trend of variation in germination of *Helminthosporium sativum* infected wheat seeds were reported by Hanson and Christensen (1953). They reported 66% and 62% seed germination having seed infection 81% and 74%, respectively with *Helminthosporium sativum.* Choudhary et al. (1984) reported that germination of the infected (black pointed) seeds both in blotter and pot soil was found to decrease by 11.6% and 16.0%, respectively. Hossain (2000) reported that maximum reduction of germination was found by 20.20 and 42.69% in blotter and rolled paper towel method, respectively in 28% black pointed seeds. Reduction in germination of wheat seeds due to black point infection was also recorded by other workers (Parashar and Chohan, 1967; Rana and Gupta, 1982; Sinha and Thapliyal, 1984, Zhang et al., 1990). Chowdhury et al. (2010) reported that germination of wheat seeds having different levels of black pointed seed ranged 69.00-97.00 and 72.67-96.67 respectively in blotter method and rolled paper towel method depending on level of seed infection in wheat seeds. Malakar (2003) found abnormal seedlings and dead seeds increased with the increase inoculums of *B. sorokiniana*. Siddique (2003) reported that the highest normal seedlings and lowest abnormal seedlings were found in clean seeds. The prevalence of higher number of black pointed seeds might be responsible for causing seed rot. From the present study, it was revealed that different levels of black pointed seed by *Bipolarissorokiniana* had significant relationship with seedling infection as well as seedling health. Seedling infection increased with the increasing level of black pointed seed in rolled paper towel method.

The result showed that the treatments significantly influenced shoot length (cm) root length (cm), seedling weight and vigor index of 7 days old seedlings (Table 8). The root length varied from 11.13 to 13.77 cm, where the highest (13.77 cm) root length was found in T_7 statistically similar with T_{13} (13.53 cm). The minimum (11.13 cm) root length was found in T_6 which was statistically similar with T_5 (11.47 cm) as well as $T_{18.}$ In case of shoot length, the highest (9.83 cm) shoot length was found in T_7 which was statistically similar with T_{13} (9.57 cm). The minimum (7.87 cm) shoot length was found in T_6 which was statistically similar with $T_{5.}$ Considering seedling weight, the highest (4.30 g) seedling weight was found in T_7 statistically similar with T_8 (4.17). The minimum (3.43g) seedling weight was found in T_6 which was statistically similar with T_5 (3.53 g) as well as preceded by T_{18}

Table 8: Effect of different levels of black pointed seed and vermicompost on root length (cm), shoot length (cm), seedling weight and vigor index in the laboratory

Treatment	Root length (cm)	Shoot Length (cm)	Seedling weight (g)	Vigor index
T_1	13.10^{a-d}	9.23^{a-d}	4.03^{a-d}	1935^{a,b}
T_2	12.73^{a-f}	8.97^{b-e}	3.92^{a-e}	1806^{b,c}
T_3	12.37^{c-g}	8.73^{c-g}	3.80^{b-f}	1626^{c-f}
T_4	11.87^{e-h}	8.33^{e-h}	3.67^{d-f}	1494^{e-g}
T_5	11.47^{g,h}	8.07^{gh}	3.53^{e,f}	1353^{g,h}
T_6	11.13^{h}	7.87^{h}	3.43^{f}	1226^{h}
T_7	13.77^{a}	9.83^{a}	4.30^{a}	2157^{a}
T_8	13.03^{a-d}	9.43^{a-c}	4.17^{a,b}	1984^{a,b}
T_9	12.83^{a-f}	9.03^{b-e}	4.03^{a-d}	1829^{b,c}
T_{10}	12.53^{b-g}	8.83^{b-f}	3.93^{a-e}	1687^{c-e}
T_{11}	12.13^{d-h}	8.50^{d-h}	3.73^{c-f}	1574^{d-g}
T_{12}	11.77^{f-h}	8.40^{e-h}	3.57 e,f	1418^{f-h}
T_{13}	13.53^{a,b}	9.57^{ab}	4.13^{a-c}	2064^{a}
T_{14}	13.23^{a-c}	9.23^{a-d}	4.03^{a-d}	1940^{a,b}
T_{15}	12.93^{a-e}	8.90^{b-e}	3.93^{a-e}	1761^{b-d}
T_{16}	12.53^{b-g}	8.73^{c-g}	3.83^{b-f}	1616^{c-f}
T_{17}	11.93^{e-h}	8.33^{e-h}	3.67^{d-f}	1463^{e-g}
T_{18}	11.47^{g,h}	8.13^{f-h}	3.53^{ef}	1346^{g,h}
LSD (0.01)	1.10	0.75	0.41	231.10

(3.53) and T_{12} (3.57). Vigor index (VI) for all the treatments differed significantly with a range of 1226 to 2157 (Table 8). The maximum vigor index (2157) was recorded in seedlings under T_7 which was statistically similar with T_{13} (2064). The minimum vigor index (1226) was counted in T_6 which was statistically similar with T_{18} (1346) and T_5 (1353). The shoot length, root length, seedling weight and also vigor index (VI) were decreased with the increasing levels of black pointed seed. The findings of the present study are supported by the earlier of Rana and Gupta (1982). They found that black point infection greatly affected root and shoot growth of the seedlings, the effect being very prominent on root growth. Rashid and Fakir (1998) reported that percent reduction in shoot and root length increased with the increase of infection grade of seed transmitted *B.sorokiniana* and the overall reductions were highest for root length. He also mentioned that the seedlings that developed from such seed were usually poor vigorous. Hossain (2000) found that the rate of reduction of growth was the maximum by 28% black pointed seeds as recorded root length was 57.21 cm and for shoot length was 41.40 cm. He also mentioned that the vigor index was found with maximum reduction (72.63%) resulted by the seedlings of 28% black pointed seeds. Chowdhury et al. (2010) found that different levels of black pointed seed greatly affected root and shoot growth of wheat seedlings where as vigor index of seedling was 1851.81, 1392.02, 1203.55, 971.16, 841.62 and 669.36.

In the view of above finding, it has been found that minimum level of black pointed seeds resulted minimum disease incidence and subsequent disease development in the field as well as for healthy seed production.

4. Conclusion

Seed sample treated with treatment vermicompost @ 1.5 t ha^{-1} and apparently healthy seed treated with Provax 200 @ 0.25%) showed promising results in respect of emergence of seedling, disease severity and seed production. However, more investigations are needed to be persued in different Agro-ecological Zones to fix up a suitable seed health standard against leaf blight of wheat (*Bipolaris sorokiniana*) for healthy seed production.

5. References

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